IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re application of:** Bauer et al.
**Application No.** 10/686,548
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**For:** POSITIVE DETECTION LATERAL-FLOW APPARATUS AND METHOD FOR SMALL AND LARGE ANALYTES
**Examiner:** Gary W. Counts
**Art Unit:** 1641
**Attorney Reference No.** 7772-66637-01

CERTIFICATE OF MAILING

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Attorney or Agent for Applicant(s) William D. Noonan

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**SECOND DECLARATION OF DR. ROBERT L. BUCK UNDER RULE 132**

1. I hold a Ph.D. in Biochemistry and have worked for 23 years in the field of medical diagnostics. I am currently a consultant for Quantrx Biomedical, and a partner at Labfx, a biotechnology company in Portland, Oregon. For many years I have been involved in research and development related to lateral flow test strips.

2. I have previously performed experiments that were reported in a May 15, 2005 Declaration under Rule 132. Those experiments demonstrated that it is not possible to determine from the disclosure of Boehringer et al. (WO 9839657) that a latex conjugate in a labeling zone and an analyte applied to a sample application area in that reference would separate before reaching the primary capture area. Since many variables enter into the separation of the conjugate and the analyte, and Boehringer did not control for those variables, that reference can not be said to disclose differential migration of the conjugate and analyte in a manner that the analyte will reach the primary capture area ahead of the conjugate. My prior declaration demonstrated, for example, that merely altering the distance between the primary capture area and the conjugate in the labeling zone will cause the conjugate and analyte to reach the primary capture area substantially simultaneously.

3. I have now obtained some additional data that illustrate that the selection of parameters that achieve differential migration provides a lateral flow competitive assay that has enhanced binding efficiency in the primary capture area and provides unexpectedly superior test results. The lateral flow assay we constructed is similar to that shown in the figures of pending U.S. Application No. 10/686,548 in which a lateral flow test strip includes (in a direction of distal flow) a sample application area, a labeling zone that contains a labeled conjugate, a primary capture area (PCA) where an analyte and the conjugate compete for binding, and a secondary capture area (SCA) that binds conjugate that is not bound at the primary capture area. If analyte is absent from a sample applied to the test strip, then conjugate can bind at the PCA and provide a visible signal in the PCA as compared to a weak or absent signal in the SCA. When increasing concentrations of analyte are present in the sample, the analyte competes with the conjugate for binding in the PCA so that the conjugate binds less completely at the PCA and unbound conjugate moves along the test strip to instead bind to the SCA. Hence increasing concentrations of analyte in the sample are indicated by progressively stronger signal (such as color intensity) at the SCA, and lesser signal (such as color intensity) at the PCA.

4. We have found that an immediate release of the conjugate from the labeling zone allows it to flow in the same liquid wave front with the analyte so that a large bolus of the conjugate arrives at the PCA, which overwhelms the binding capacity of the PCA and results in poor binding efficiency of the conjugate at the primary capture area. Poor binding of the conjugate bolus results in "leak through" of the conjugate to the SCA, which is very problematic because it provides a false positive result in the assay. For example, even if no analyte is present in the sample, the poor binding of the bolus to the PCA allows an undesired amount of conjugate to reach the SCA and provide a signal that can incorrectly indicate the presence of analyte in the sample. In addition, the rapid release of conjugate from the labeling zone allows the conjugate to reach the PCA at about the same time as the free analyte and diminishes the amount of time the analyte has to bind to the PCA. This reduced time for binding of the analyte to the PCA results in less conjugate being displaced from the SCA, which in turn results in less signal generation in the SCA. The reduction in displacement interferes with the ability of the assay to provide a

progressively more intense signal from the SCA (as compared to the PCA) as the concentration of free analyte increases.

5. These principles were illustrated with an amphetamine assay using the following materials:

Latex conjugate dilution buffer (LCDB)
Millipore blocked glass fiber conjugate release pad (MCRP)
Whatman blocked glass fiber conjugate release pad (WCRP)
Cassette housing for lateral flow test strips
Amphetamine (AMP) test strips containing an anti-AMP in the PCA, Goat anti-mouse IgG (GAM) in the SCA, and sample and absorption pads were pre-laminated to backing
Amphetamine solutions in PBS with 250 µg/ml blgG
Digital camera and computer

6. The experiments were performed by spraying the test strip with a PCA line of mouse monoclonal anti-amphetamine antibody at a concentration of 1.0 mg/ml at a rate of 1.0 µl/cm. The SCA consisted of a line of Goat anti-mouse IgG sprayed at the same concentration and rate as the PCA. The conjugate was prepared as a mixture of BSA-amphetamine conjugate and mouse IgG covalently attached to the carboxyl-derivative blue latex.

a. MCRP with un-sonicated latex (MCRP-U)

b. WCRP with un-sonicated latex (WCRP-U)

The Millipore conjugate release pad (MCRP) had been empirically found to release this conjugate less quickly than the Whatman conjugate release pad (WCRP), hence the Millipore pad was used to demonstrate relatively delayed release of the conjugate from the pad. This difference in how quickly the different pads release the conjugate was determined empirically by experimental observation, and is believed to be due to differing binders in the Millipore pad compared to the Whatman pad.

7. The following tables illustrate net intensities of color from the blue latex label in the PCA and SCA at 6 minutes and 24 hours after the test began. The data illustrate that the delayed release of the conjugate from the Millipore pad as compared to the immediate release from the Whatman pad resulted in a much greater reduction of binding of conjugate at the PCA as concentrations of free analyte increased. Hence delayed release of the conjugate from the Millipore pad produced a test in which the colored signal from the PCA is substantially reduced

in the presence of free analyte, thereby providing a superior test that is less confusing to the user. This reduced signal from the PCA is believed to be a function of the delayed arrival of the conjugate at the PCA which improves the efficiency of binding of free analyte at the PCA and increases displacement of conjugate from the PCA to the SCA when free analyte is present in the sample.

6 minutes net intensities

| AMP conc | | MCRP-U | | WCRP-U |
|---|---|---|---|---|
| 0 | PCA | 24.28 | | 20.25 |
| | SCA | 8.00 | | 7.69 |
| | | | | |
| 2.5 | PCA | 2.54 | | 6.89 |
| | SCA | 8.32 | | 11.72 |
| | | | | |
| 5 | PCA | 0.94 | | 6.53 |
| | SCA | 9.50 | | 16.67 |

24 hours net intensities

| AMP conc | | MCRP-U | | WCRP-U |
|---|---|---|---|---|
| 0 | PCA | 29.71 | | 18.28 |
| | SCA | 15.73 | | 9.98 |
| | | | | |
| 2.5 | PCA | 4.15 | | 9.93 |
| | SCA | 18.70 | | 16.99 |
| | | | | |
| 5 | PCA | 1.66 | | 7.11 |
| | SCA | 13.61 | | 16.65 |

8. The following table reports the ratio (PCA/SCA) of total amounts of conjugate at the PCA and SCA at 6 minutes and 24 hours after the test began.

| | | PCA/SCA | | |
|---|---|---|---|---|
| AMP conc | 6 min | 24 hr | 6 min | 24 hr |
| | MCRP-U | MCRP-U | WCRP-U | WCRP-U |
| 0 | 3.035 | 1.888748 | 2.63329 | 1.831663 |
| 2.5 | 0.305288 | 0.221925 | 0.587884 | 0.584461 |
| 5 | 0.098947 | 0.121969 | 0.391722 | 0.427027 |

The data in this table shows that delayed release of the conjugate from the pad improves both primary binding and displacement of conjugate from the PCA by free analyte. In the absence of free analyte (0) at 6 minutes (which would be a time similar to the period of time the test strip would be allowed to develop in clinical or home use), the amount of conjugate in the PCA as compared to the SCA (expressed as PCA/SCA) was higher for delayed release of conjugate (MCRP) as compared to the non-delayed release of conjugate (WCRP). As the concentration of free analyte increases, the ratio of PCA/SCA is less for the delayed release situation (MCRP) as compared to the immediate release situation (WCRP), as would be desired for a positive test result in which the signal from the SCA is greater than from the PCA.

9. The unexpected superiority of the delayed release of conjugate from the pad is further illustrated in the following table, which reports percent displacement of conjugate from the PCA at 6 minutes and 24 hours, with the delayed (MCRP) and immediate release (WCRP) of conjugate, for amphetamine concentrations of 0, 2.5 and 5 ng/ml.

| | | %displacement | | |
|---|---|---|---|---|
| AMP conc | 6 min | 24 hr | 6 min | 24 hr |
| | MCRP-U | MCRP-U | WCRP-U | WCRP-U |
| 0 | 0 | 0 | 0 | 0 |
| 2.5 | 89.94107 | 88.25014 | 77.67493 | 68.09122 |
| 5 | 96.73979 | 93.54233 | 85.12425 | 76.68638 |

Delayed release of the conjugate from the pad (MCRP) provided higher percentages of displacement of conjugate from the PCA as compared to immediate release of the conjugate (WCRP) as the concentration of free analyte increased.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

By Robert L. Buck
Robert L. Buck, Ph.D.

Date 12/4/06